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厦 门 大 学

硕 士 学 位 论 文

骨髓间充质干细胞向肝星状细胞  
定向分化的研究

Studies of Differentiation of Mesenchymal Stem Cells into  
Hepatic Stellate Cells

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专 业 名 称: 生物化学与分子生物学

论文提交日期: 2010 年 4 月

论文答辩时间: 2010 年 6 月

学位授予日期:

答辩委员会主席：\_\_\_\_\_

评 阅 人：\_\_\_\_\_

2010 年 5 月

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## 摘 要

### 背景和目的

研究表明,肝星状细胞(HSCs)在激活后有极强的细胞免疫抑制功能,将自体的 HSCs 与同种异体胰岛细胞共同移植后,由于其有效的免疫抑制作用和分泌细胞外基质在被移植物外产生包膜的生物学特性,对胰岛移植细胞的长期存活具有重要的作用。因此, HSCs 在保护胰岛细胞移植上有着极高的临床应用价值。但由于 HSCs 获取困难,从而阻碍了其在临床上的应用。骨髓间充质干细胞(BMSCs)具有取材容易、体外增殖能力强、可分化为不同类型成熟细胞并用于多种疾病治疗等特点,使我们找到了 HSCs 在临床应用上的途径。为证实假设,本实验设计不同条件探讨将 BMSCs 诱导分化为 HSCs 的可能性。

### 研究方法

采用全骨髓贴壁培养法分离纯化 BMSCs,光学显微镜观察细胞形态,流式细胞术检测细胞周期和表面分子,成脂成骨专用诱导培养基检测 BMSCs 多向分化潜能,混合淋巴细胞反应(MLR)检测 BMSCs 的免疫调节功能;采用原位灌注消化和密度梯度离心法分离获得 HSCs,光学显微镜观察细胞形态,免疫荧光技术检测特异分子 desmin 和  $\alpha$ -SMA 的表达;采用 Transwell 构建间接共培养体系,将 BMSCs 分别与下列三组共培养:(1)被激活的 HSCs;(2)被激活的 HSCs 同时加入 1ng/ml 的 IL-10;(3) 1ng/ml 的 IL-10。诱导后对此三组分别用流式细胞术、MLR、免疫荧光和 ELISA 等方法进行生物学性质鉴定。

### 研究结果

成功分离出 BMSCs,呈均一梭形,旋涡状生长。流式细胞术检测显示细胞高表达 CD29、CD34、CD44、CD105,低表达 CD11b、CD14、CD45、CD73,不表达共刺激分子 CD40、CD54、CD80、CD86、MHC II 和 PDL-1,经 IFN $\gamma$  刺激后共刺激分子 CD54、CD80、MHC II 和 PDL-1 表达水平上调。MLR 结果表明 BMSCs 具有免疫抑制功能。BMSCs 有多向分化潜能,体外能诱导分化为成骨细胞和脂肪细胞。免疫荧光显示 BMSCs 不表达 desmin 和  $\alpha$ -SMA。成功分离出 HSCs,激活状态下免疫荧光实验显示表达  $\alpha$ -SMA 和 desmin。间接共培养的 3 组中,单纯与 HSCs 或 IL-10 共培养的 1、3 两组 BMSCs 免疫抑制功能及胶原分泌检测结果与诱导前无显著差异。而在 HSCs 和 IL-10 共同作用下与 BMSCs 间

接培养的第 2 组, 诱导后的 BMSCs 免疫抑制功能明显增强, 并呈现出活化的 HSCs 的生物学特征, 如 desmin 和  $\alpha$ -SMA 阳性表达。将诱导后的 BMSCs 再扩增, 上清中通过 ELISA 检测表明 I 型胶原分泌与活化的 HSCs 分泌量无统计学差异。

## 研究结论

成功分离了 BMSCs 和 HSCs, 两者在有 IL-10 细胞因子作用的条件下间接共培养后, 被诱导的 BMSCs 表现出了与 HSCs 相似的一些特性, 为后续体内胰岛细胞移植提供了有利的实验基础。

**关键词:** 间充质干细胞; 肝星状细胞; 分化

## Abstract

### Background and Objective

Research showed that activated hepatic stellate cells (HSCs) indicated strongly inhibition of immunity, because of this and secretion of extracellular matrix producing coat outside the transplanted cell, transplantation of allogeneic islet cells with HSCs could induce islet cells long-term survival, so, HSCs has highly clinical valuable in islet transplantation. However, HSCs are difficult to obtain, this hinder the application of clinical. Bone marrow mesenchymal stem cells (BMSCs) can easily to separate, quickly to increase, possibly to differentiate into multiple lineages. In addition, due to their limited immunogenicity, BMSCs are currently under investigation for their possible use to treat immuno-mediated diseases. Because these characteristics, it makes me find a way to clinical application. To prove hypothesis, this experiment designs different conditions to investigate the possibility of BMSCs differentiation into HSCs.

### Methods

BMSCs were isolated by using the differentiate adherence method and expansion in culture. The resulting BMSCs were used for our studies. To confirm the identity of BMSCs, BMSCs were detected from the morphology, phenotype, differentiation capacity and immunogenicity. Primary HSCs cells were freshly isolated from mice liver nonparenchymal cells. The liver was perfused with collagenase and pronase solution. HSCs were isolated by density gradient centrifugation and adhesion culture. HSCs were detected from the specific protein desmin and  $\alpha$ -SMA using immunofluorescence method. By using transwell to establishment of indirect coculture system, investigation of the effects of HSCs in different condition on the BMSCs, the different condition including: (1)activated HSCs; (2)activated HSCs and 1ng/ml IL-10; (3)1ng/ml IL-10. After differentiation, BMSCs were detected from the morphology, phenotype, collagen secretion and immunogenicity.

## Results

BMSCs expanded in vitro exhibited a fibroblast-like morphology. BMSCs were high positive expression of CD29, CD34, CD44 and CD105, low positive expression of CD11b, CD14, CD45, CD73 and negative expression of CD40, CD54, CD80, CD86, MHC II and PDL-1 by flow cytometry. When IFN $\gamma$  stimulation can up-regulate the expression of CD54, PDL-1, MHC-I and MHC-II. BMSCs were induced to differentiate into osteoblasts and fat cells by using special culture media. In MLR, BMSCs failed to elicit a response from T cells. BMSCs suppressed the MLR and the restrain ability was dose dependent. BMSCs were not expression of  $\alpha$ -SMA and desmin by immunofluorescence method. HSCs expanded in vitro exhibited a fibroblast-like morphology. Activated HSCs were expression of  $\alpha$ -SMA and desmin. Indirect co-culture group 2, in HSCs and common role with IL-10, BMSCs significantly enhanced immune inhibition function, expressed higher immunological suppression. Up-regulated the expression of CD54, PDL-1 and showed biological characteristics of activated HSCs, such as desmin and  $\alpha$ -SMA were expression. After the induced BMSCs further proliferation, the type I collagen secretion was no significant difference with activated HSCs. But group 1 and 3 were not significant differences with before induced.

## Conclusion

Successful separation of BMSCs and HSCs, both in a role of cytokines IL-10 under the conditions of indirect co-cultivation, BMSCs showed some similar characteristics with HSCs. For subsequent pancreatic islets transplantation in vivo experiments provided a favorable basis.

**Key Words:** Mesenchymal stem cells; Hepatic stellate cells; Differentiation

## 缩略词表

英文缩写	英文全称	中文全称
MSCs	mesenchymal stem cells	间充质干细胞
BMSCs	bone mesenchymal stem cells	骨髓间充质干细胞
HSCs	hepatic stellate cells	肝星状细胞
DCs	dendritic cells	树突状细胞
PBS	phosphate buffered saline	磷酸盐缓冲液
FCS	fetal calf serum	胎牛血清
DMSO	dimethylsulphoxide	二甲基亚砷
CD	cluster of differentiation	分化簇
PE	phycoerythrin	藻红蛋白（天然荧光素）
FITC	fluorescein isothiocyanate	异硫氰酸荧光素
DAPI	4',6-diamidino-2-phenylindole	4',6-二脒基-2-苯基吲哚
MHC	major histocompatibility complex	主要组织相容性复合体
PI	propidiumiodide	碘化丙啶
MLR	mixed lymphocyte reaction	混合淋巴细胞试验
LPS	lipopolysaccharide	脂多糖
IL-4	Interleukin-4	白细胞介素 4
IFN- $\gamma$	Interferon- $\gamma$	干扰素- $\gamma$
IL-10	Interleukin-10	白细胞介素 10
GM-CSF	Granulocyte-macrophage colony-stimulating factor	粒-巨噬细胞集落刺激因子



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